Effect of external mass transfer on activation energy of butyl oleate ester synthesis using a whole cell biocatalyst

Shabnam Shahhoseini, Bahram Nasernejad and Farzaneh Vahabzadeh*

Department of Chemical Engineering, Amir kabir University of Technology (Tehran Polytechnic), Tehran, Iran.

(Received 9 October 2015, Accepted 31 January 2016)

Abstract

In the present research, synthesis of butyl oleate ester from oleic acid and butanol using loofa-immobilized Rhizopus oryzae as a whole cell biocatalyst (LIC) was studied in which hexane was used as the hydrophobic solvent. Decrease of mass transfer limitations as result of the interface formation between the two immiscible substrates, positively affected on the reaction progress (87% as the ester product yielded within 10 h). By applying Arrhenius equation, the activation energy of the ester synthesis was determined as E_a=18.2 kJ/mol within temperature range of 15-45°C. It was notable to test appearance of the nonlinearity in Arrhenius plot which was indicative of presence of two sections. The reaction limited region was 15-35°C; E_a=27 kJ/mol and diffusion limited region was >35°C; E_a=6.8 kJ/mol. Eventually, in this research, influence of external mass transfer on activation energy with reference to the catalytic role of the LIC in the ester synthesis was discussed.

Keywords

Butyl oleate;
Ester synthesis;
Loofa sponge;
Activation energy;
Arrhenius equation.

1. Introduction

Use of lipases (glycerol esters hydrolysis, E.C.3.1.1.3) is mainly for hydrolysis of lipidic substrates while by changing the reaction environment from aqueous to non-aqueous, the fate of lipase action changes so that the catalysis moves away from the hydrolysis and esertification a fatty acid and an alcohol becomes dominant reaction. Applications of materials produced through biotechnological routes are diverse and preferences of these bio-products over chemically formed products are straight forward: mild reaction conditions and eco friendliness. Thus bioconversions are economically favorable processes. However the cost of enzymes restricts their industrial usages and this character has directed attentions towards the microbial cells and their potentiality as been used as the whole cell biocatalyst(s). Loofa as a natural lignocellulosic fiber is in a suitable position for the cells immobilization. The high porosity, low density, and high specific surface area are some of the interesting physical properties that along with the chemical composition of this biomaterial, have given unique character to the loofa sponge [1, 2].

Control of homogeneous catalytic reaction progression has been mainly described in terms of the

* Corresponding Author.
Tel: +98 21 64543161; Fax: +98 21 66405847;
E-mail address: far@aut.ac.ir

reaction mechanism and the rate of the conversion has been kinetically determined. While in the heterogeneous catalysis, the transfer of the involved materials from the bulk fluid phase, and the catalyst particles as well as the porous surfaces of the catalysts to the bulk, affects on the reaction extent [3, 4, 5]. Mass transfer limitations of these diffusion processes can be either external or internal where, in the latter case presence of pores and their distributions throughout the catalyst spaces are dominant factors. Mass transfer mechanisms are closely related to reactant and products molecule- molecule interactions and also collisions of the molecules with walls of the pores.

The aim of the present work was to study application of the Arrhenius equation for activation energy calculation of the ester synthesis reaction using the loofa-immobilized *Rhizopus oryzae* (lipase producer fungus) as the whole cell biocatalyst (LIC). The temperature dependence of the reaction in terms of activation energy (E_a) then was determined considering quantitative relationship between E_a and the external mass transfer when the internal diffusion was negligible.

2. Materials and method

2.1. Microorganism and media

All experiments were carried out using the filamentous fungus *R. oryzae* (PTCC 5174) as the lipase producer. The *Rhizopus oryzae* was purchased from "Persian Type Culture Collection (PTCC)" and then was routinely maintained on an agar slant made of 4% potato dextrose agar and 2% agar. The basal medium contained: 70 g polypepton; 1.0 g $NaNO_3$; 1.0 g KH_2PO_4; and 0.5 g $MgSO_4 \cdot 7H_2O$ per liter of distilled water. Olive oil as sole carbon source was added to the medium at a concentration of 30 g/l. The pH of the medium was initially adjusted to 5.6 and then allowed to follow its natural course [6].

2.2. Immobilization of cells

Loofa sponge was used as the immobilization structure for the *Rhizopus oryzae* fungi in this study. These structures were prepared in the following steps. First the loofa was cut in the form of disks with a diameter of 1.5 cm, then boiled in distilled water for 10 minutes and dried at 80 degrees Celsius in an oven. Next, 6 piece of these disks were put in an Erlenmeyer flask (250 ml) containing 50 ml of the basal medium and after sterilization, spores were inoculated from a fresh slant to the flask. The temperature during cultivation was controlled at 30 degrees Celsius on a reciprocal shaker at 150 rpm for 48 hours. In the final step, the LICs were separated from the broth and washed with tap water followed by acetone and dried under vacuum for 72 hours [7].

2.3. Experimental procedure for the enzymatic synthesis

The reactions were performed in screw-capped flasks with a working volume of 10 ml containing two substrate (oleic acid and butanol) dissolved in n-hexane. The reaction mixture was incubated at different temperatures in range of 15-55˚C, in presence of two loofa pieces containing 200 mg of *R-oryzae* cells with shaking at 250 rpm.

2.4. Analytical methods

Aliquots of the reaction mixture were withdrawn periodically and the residual acid content was assayed by the colorimetric method with use of cupric acetate-pyridine aqueous solution as the color reagent [8]. The absorbance of samples was measured at wavelength of 715 nm using a JASCO V-550 spectrophotometer. The percentage conversion in ester synthesis was based on the amount of acid consumed.

2.5. Calculation of activation energy

It is found experimentally that the rate constants (k) for many chemical reactions follow the Arrhenius equation.

$$k = A\exp(-Ea/RT)$$

or equivalently: $\ln(k) = \ln(A) - (Ea/RT)$

Where A is the pre-exponential factor and Ea is the activation energy and R is the universal gas constant. These parameters will be determined from experimental data of rate (initial reaction rate 'v' rather than k) by plotting ln(v) against 1/T [9]. This is known as an Arrhenius plot, and has an intercept of ln(A) and a slope of -Ea/R. For most reactions, the Arrhenius equation works fairly well over at least a limited temperature range.

3. Results and discussion

Synthesis of the butyl oleate ester was carried out in the temperature range of 15 to 55°C using loofa immobilized *R. oryzae* (LIC). The results presented in Fig. 1 show that, both the yield of the ester production and the initial rate of the synthesis reaction, were increased with increasing temperature in the range of 15 to 35°C (the yield changed from 66 to 83% and variation of the synthesis rate

changed from 0.89 to 1.89 mM/min). However, in the temperature range from 35 to 45 °C, the final yield of the ester production was reduced by 20% and reached to the value of 62%; and the change of reaction rate was not considerable and it remained almost constant. Sharp decrease in the reaction rate after 45°C is also seen in Fig. 1. Temperature dependence of *R. oryzae* lipase has found to be considerable above 35°C while the inactivation at 45°C was noticeable [10, 11]. The decrease of the reaction rate here in the present work began at 45 °C. It appears that loofa sponge used for the cell immobilization in the present study, has affected positively on the *R. oryzae* lipase performance.

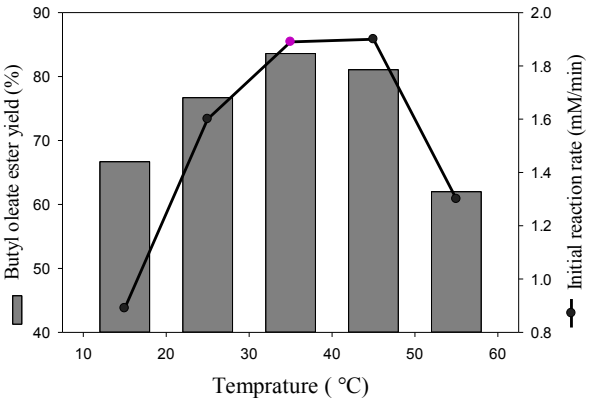

Figure 1. Yield of the butyl oleate ester as a function of temperature. Dependence of the initial reaction rate of the ester synthesis on the temperature is also shown. Reaction conditions were as follows: oleic acid to butanol molar ratio 1:1, agitation speed 250 rpm, 10 ml was the total volume of the reaction mixture. The cell content covered on two pieces of LIC was 200 mg and hexane was the reaction solvent.

Further work was to examine temperature dependence (15-45°C) of the reaction rate in terms of Arrhenius equation and the result is shown in Fig.2. Appearance of nonlinearity in the Arrhenius plot as seen in the inset of Fig. 2 indicates that behavior of the test reaction can be described in terms of two regions: the diffusion limited (>35°C; expressing E_a=6.8 kJ/mol) and the reaction limited (lower section of the plot or 15 to 35°C; expressing E_a=27 kJ/mol). Regression technique was used to treat all the experimental points from 15 to 55°C as seen in Fig.2. Activation energy obtained from the regression line passing from all points then was 18.4 kJ/mol. Complexes of enzyme-bound substrate affect on the enzyme's conformation and increase of the shape distortion increases the lability of the bounds to be broken. Facilitation of the bound cleavage through the catalytic action of the enzyme

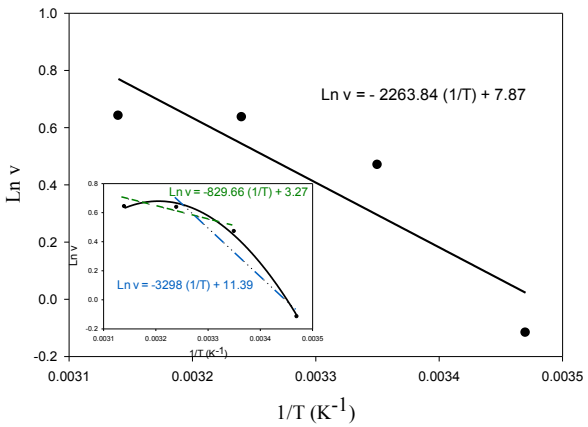

Figure 2. Arrhenius plot for butyl oleate ester synthesis using loofa immobilized Rizopus oryzae as the whole cell biocatalyst (LIC). Details of the reaction mixture are given in Fig. 1.

is a way, to lower the activation energy of the reaction. The reported E_a value is comparable with the activation energies for the lipases (Table 1).

The nonlinearity seen in Arrhenius plot (Fig. 2 inset) indicates presence of two sections. Control of the homogeneous catalytic reaction progression is described in terms of reaction mechanism and

Table 1. A brief summary of the activation energy for the some reactions catalyzed by lipase.

Biocatalyst / Lipase source	Acyl donor	T (ºC)	E_a (kJ/mol)	Ref.
Lipozym TL IM	palm oil	40-65	16.85	[12]
Novozym 435	oleic acid	30-45	25.6	[13]
Aspergillus niger	gallic acid	30-47.5	23.29	[14]
Novozym 435	cottonseed oil	25-50	19.2	[15]

the rate of the conversion which kinetically can be determined. In a heterogeneous catalysis, movement of the involved substrates molecules and also products between the bulk phase and the enzyme surface (LIC) all have considerable effects on the reaction extent (i. e., mass transfer effects limit the diffusion process). With reference to the *R. oryzae* distribution on the loofa pieces, one may say that resistances of the involved molecules to external diffusion are dominant. The diffusion limited regime for the ester synthesis in the present work is observable in the inset of Fig. 2 which was determined as E_a = 6.8 kJ/mol.

Obtaining the activation energy for the enzymatic reactions using Arrhenius equation should be performed in the range of low temperature where drawing a true straight line for Ln (v) as a function of 1/T, is perfectly possible [5, 9].

4. Conclusions

Temperature dependence of the butyl oleate ester synthesis showed that the reaction kinetic is explainable at the low temperature. Industrial usages of the enzymes highly depend on understanding of the involved mechanism in any the particular conversion. More works are needed to describe the details of the LIC catalytic action in synthesis of butyl oleate ester.

References

1. Saeed, A. and Iqbal, M. (2013). "Loofa (Luffa cylindrica) Sponge: Review of Development of the Biomatrix as a Tool for Biotechnological Applications". *Biotechnol. Prog*, Vol. 29, No. 3, pp. 573-600.

2. Saw, S.K., Purwar, R., Nandy, S., Ghose, J. and Sarkhel, G. (2013). "Fabrication, Characterization, and Evaluation of Luffa cylindrica Fiber Reinforced Epoxy Composites". *Bioresources*, Vol. 8, No. 4, pp. 4805-4826.

3. Mukherjea, R.N., Bhattacharya, P., Gangopadhyay, T. and Ghosh, B.K. (1980). "Effect of External Mass Transfer on Activation Energy of Hydrolysis of BAEE and Casein by Trypsin Immobilized on Molecular Sieve Type 4A". *Biotechnology and Bioengineering*, Vol. XXII, pp. 543-553.

4. Davis, M.E. and Davis, R.J. (2003). "*Fundamentals of chemical reaction engineering*", first edition, McGraw-Hill Higher.

5. Bailey, J.E. and Ollis, D.F. (1986). "*Biochemical Engineering Fundamentals*". Second Edition, McGraw Hill Higher, New York.

6. Ban, K., Kaieda, M., Matsumoto, T., Kondo, A. and Fukuda, H. (2001). "Whole cell biocatalyst for biodiesel fuel production utilizing *Rhizopus oryzae* cells immobilized within biomass support particles". *Biochemical Engineering Journal*, Vol. 8, pp. 39-43.

7. Sattari, S., Vahabzadeh F. and Aghtaei, H.K. (2015). "Performance of loofa-immobilized *Rhizopus oryzae* in the enzymatic production of biodiesel with use of oleic acid in n-hexane medium". *Brazilian Journal of Chemical Engineering*, Vol. 32, No. 02, pp. 367-376.

8. Lowry, R.R. and Tinsley, I.J. (1976). "Rapid colorimetric determination of free fatty acids". *J. Am. Oil Chem. Soc.*, Vol. 53, pp. 470-472.

9. Fogler, H. S. (2006). "*Elements of Chemical Reaction Engineering*". Fourth Edition, Pearson Education, Inc.

10. Hiol, A., Jonzo, M. D., Rugani, N., Druet, D., Sarda, L. and Comeau, L. C. (2000). "Purification and characterization of an extracellular lipase from a thermophilic *Rhizopus oryzae* strain isolated from palm fruit". *Enzyme and Microbial Technology*, Vol. 26, pp. 421-430.

11. Ben Salah, R., Ghamghui, H., Miled, N., Mejdoub, H. and Gargouri, Y. (2007). "Production of butyl acetate ester by lipase from novel strain of *Rhizopus oryzae*". *Journal of Bioscience and Bioengine*, Vol. 103, No. 4, pp. 368-372.

12. Basri, M., Kassim, M.A., Mohamad, R. and Ariff, A.B. (2013). "Optimization and kinetic study on the synthesis of palm oil ester using Lipozyme TL IM". *Journal of Molecular Catalysis B: Enzymatic*, Vol. 85-86, pp. 214-219.

13. Hajar, M. and Vahabzadeh, F. (2016). "Production of a biodiesel additive in a stirred basket reactor using immobilized lipase: Kinetic and mass transfer analysis", *Korean J. Chem. Eng.*, DOI: 10.1007/s11814-015-0249-x.

14. Yu, X.W. and Li, Y.Q. (2006). "Kinetics and thermodynamics of synthesis of propyl gallate by

mycelium-bound tannase from Aspergillus niger in organic solvent". *Journal of Molecular Catalysis B: Enzymatic*, Vol. 40, pp. 44-50.

15. Royon, D., Daz, M., Ellenrieder, G. and Locatelli, S. (2007). "Enzymatic production of biodiesel from cotton seed oil using t-butanol as a solvent". *Bioresource Technology*, Vol. 98, pp. 648-653.